METHODS OF PROMOTING CNS NEURONAL REPAIR BY INHIBITING LRP-1

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 15/189,965, filed on Jun. 22, 2016, now U.S. Pat. No. 10,829,536, issued Nov. 10, 2020, which is a continuation of U.S. patent application Ser. No. 14/113,626, filed on Feb. 18, 2014, now U.S. Pat. No. 9,376,481, issued Jun. 28, 2016, which is a 35 U.S.C. § 371 National Stage of International Application No. PCT/US2012/035125, filed Apr. 26, 2012, and claims the benefit under 35 U.S.C. § 119(e) of U.S. Patent Application No. 61/479,210, filed on Apr. 26, 2011, the content of each of which is hereby incorporated herein by reference in their entirety for all purposes.

STATEMENT OF GOVERNMENTAL SUPPORT

[0002] This invention was made with government support under NS054671 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Jun. 1, 2021, is named 114198-2024_SeqListing_ST25.txt and is 85,443 bytes in size.

FIELD OF THE INVENTION

[0004] The present invention relates to reducing or inhibiting the function and/or signaling through the low density lipoprotein receptor-related protein-1 (LRP-1) to promote, enhance and/or restore neuron regeneration and/or nerve growth in the presence of injury to the CNS, e.g., to counteract CNS damage resulting from spinal cord injury or traumatic brain injury.

BACKGROUND OF THE INVENTION

[0005] Recovery from CNS injury is limited by macromolecules that accumulate in the micro-environment of damaged neurons and inhibit axonal regeneration (Berry (1982). *Bibliotheca anatomica,* 1-11; Ng, et al., (1996). *Brain Res* 720:17-24; Yiu and He, (2006). Nat Rev Neurosci 7:617-627). In the acute phase of CNS injury, myelin-derived proteins are principally responsible for regenerative failure. The inhibitory proteins include myelin-associated glycoprotein (MAG) (Tang, et al., (1997). *Mol Cell Neurosci* 9:333-346), oligodendrocyte myelin glycoprotein (OMgp) (Wang, et al., (2002). *Nature* 417:941-944), and Nogo (Fournier, (2001) *Nature* 409:341-346; Filbin, (2003). *Nat Rev Neurosci* 4:703-713). Later in the course of CNS injury, chondroitin sulfate proteoglycans (CSPGs) in the glial scar inhibit axonal regeneration (Oohira, et al., (1991). *J Neurosci* 11:822-827.; Hynds and Snow, (1999). *Experimental Neurology* 160:244-255). MAG, OMgp, and Nogo bind to the neuronal receptors, Nogo-66 receptor (NgR1) (Fournier et al., 2001, supra) and paired immunoglobulin-like receptor B (PirB) (Atwal et al., (2008). *Science* 322:967-970.). MAG also binds gangliosides which might play a role in inhibition (Vyas, et al., *Proc Natl Acad Sci USA.* 2002 Jun. 11; 99(12):8412-7). Co-receptors, including p75NTR (Wong et al., (2002). *Nat Neurosci* 5:1302-1308), Nogo-66 receptor 1 (NgR1) and LINGO1 (Mi et al., (2004). *Nat Neurosci* 7:221-228), are recruited into the Nogo receptor complex and neuronal signaling to RhoA is initiated (Yamashita et al., (2002). *J Cell Biol* 157:565-570). In certain instances, TAJ/TROY binds to NgR1 and can replace p75NTR in the p75NTR/NgR1/LINGO-1 complex to activate RhoA in the presence of myelin inhibitors (Shao, et al., *Neuron* (2005) 45(3):353-9). Activated RhoA causes growth cone collapse and inhibits neurite outgrowth (Kozma et al., (1997). *Mol Cell Biol* 17:1201-1211; Kuhn et al., (1999). *J Neurosci* 19:1965-1975; Madura et al., (2004). *EMBO Reports* 5:412-417).

[0006] Low density lipoprotein receptor-related protein-1 (LRP1) is a type-1 transmembrane receptor that binds over forty structurally and functionally distinct ligands, mediating their endocytosis and delivery to lysosomes (Strickland et al., (2002). *Trends Endocrinol Metab* 13:66-74). LRP1 also functions in phagocytosis of large particles, including myelin vesicles (Lillis et al., (2008). *J Immunol* 181:364-373; Gaultier et al., (2009). *J Cell Sci* 122:1155-1162). Neurons in the CNS and PNS express LRP1 (Wolf et al., (1992). *Am J Pathol* 141, 37-42; Bu et al., (1994). *J Biol Chem* 269:18521-18528; Campana et al., (2006). *J Neurosci* 26:11197-11207). At the subcellular level, LRP1 has been localized in dendritic shafts and spines, consistent with its known ability to interact with post-synaptic density proteins and regulate long-term potentiation (Brown et al., (1997). *Brain Res* 747:313-317; May et al., (2004). *Mol Cell Biol* 24:8872-8883) and in neuronal growth cones, both in intercellular vesicles and at the cell surface (Steuble et al., (2010). *Proteomics* 10:3775-3788).

[0007] In neurons and neuron-like cell lines, binding and endocytosis of specific LRP1 ligands is coupled with activation of cell-signaling (Qiu et al., (2004). *J Biol Chem* 279:34948-34956; Hayashi et al., (2007). *J Neurosci* 27:1933-1941; Fuentealba et al., (2009). *J Biol Chem* 284: 34045-34053; Mantuano, et al., (2008). *J Neurosci* 28:11571-11582; Shi et al., (2009). *Sci Signal* 2:ra18). Src family kinases (SFKs), which are activated downstream of LRP1, transactivate Trk receptors, accounting mechanistically for the ability of LRP1 ligands to induce neurite outgrowth (Shi et al., 2009, supra). However, LRP1 also regulates cell-signaling by serving as a co-receptor or by regulating the trafficking of other receptors, such as uPAR, TNFR1, and PDGF receptor (Webb et al., (2001). *J Cell Biol* 152:741-752; Boucher et al., (2003). *Science* 300:329-332; Gaultier et al., (2008). Blood 111:5316-5325). The function of LRP1 in conjunction with other cell-signaling receptors explains the activity of LRP1 in regulation of inflammation, atherogenesis, and cell growth.

[0008] Our previous work demonstrating myelin phagocytosis by LRP1 (Gaultier et al., (2009). *J Cell Sci* 122: 1155-1162) prompted us to examine the role of LRP1 in pathways by which myelin-associated proteins inhibit axonal regeneration. We demonstrate that LRP1 is an endocytic receptor for myelin-associated inhibitory proteins, including e.g., MAG, OMgp, and Nogo isoforms. Binding of MAG to LRP1 recruits p75NTR into complex with LRP1. Both p75NTR and LRP1 are required for RhoA activation and inhibition of neurite outgrowth. Multiple strategies for inactivating LRP1 were effective at reversing the effects of MAG and purified myelin on neurite outgrowth. Our results